Supplemental Figure Legends

Figure S1, related to Figure 1. Origin of strains and primary neuron seeding
(A) Diverse sources of aggregated tau were used to derive 18 putative tau strains in a monoclonal HEK293 cell line (Clone 1/DS1). Strains were derived from different recombinant protein, transgenic mouse, and patient sources as indicated in the table. Some strains were passaged from cell lines previously characterized. Strains are color-coded based on their associated inclusion morphology.

(B) Limited proteolysis of DS1, 4, 6, 7, 9, 10 and 11 demonstrates the digestion patterns are stable even upon dilution with HEK lysate. Strain lysate diluted 1:1 with HEK lysate is indicated with a D.

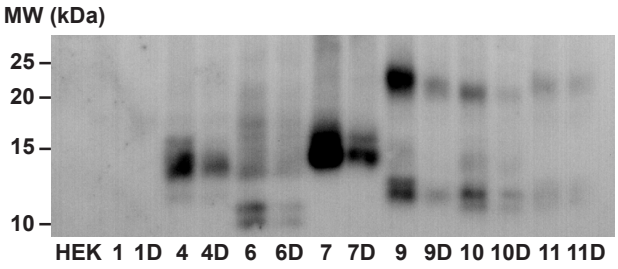
(C) Strains showed variability in their ability to seed the formation of insoluble tau aggregates in primary hippocampal neurons expressing full-length 1N4R tau(P301S)-YFP. Lysates from individual strains were added to primary neurons. Eleven days later, neurons were fixed with Triton X-100 extraction of soluble material. Accumulation of conformationally altered tau was assessed by MC1 staining (red). Strong seeders in the tau split-luciferase complementation assay produced more insoluble tau pathology in neurons. Scale bars = 50 μm for the wide view and 10 μm for the inset images.

A

Strain (DS)	Morphology	Origin of Inoculate	Passaged Line From Sanders et al. (2014)
1	Diffuse	None	Clone 1
2	Mosaic	AGD Cell Line	AGD2 (Mosaic)
3	Ordered	Rec Fibrils	N/A
4	Speckles	AD Brain Homogenate	N/A
5	Speckles	Rec Fibrils	N/A
6	Threads	P301S Mouse Homogenate	N/A
7	Speckles	Rec Fibrils	N/A
8	Speckles	P301S Mouse Homogenate	N/A
9	Speckles	Rec Fibrils	Clone 9
10	Ordered	Rec Fibrils	Clone 10
11	Disordered	CBD Cell Line	CBD3 (Disordered)
12	Speckles	CBD Cell Line	CBD3 (Speckles)
13	Disordered	CBD Cell Line	CBD5 (Disordered)
14	Ordered	Rec Fibrils	N/A
15	Threads	Rec Fibrils	N/A
16	Speckles	CBD Cell Line	CBD3 (Speckles)
17	Speckles	AD Cell Line	AD1 (Speckles)
18	Speckles	CTE Brain Homogenate	N/A
19	Ordered	Rec Fibrils	N/A

Abbreviations:
Rec fibrils = recombinant tau fibrils
AGD = argyrophilic grain disease
AD = Alzheimer's disease
CBD = corticobasal degeneration
CTE = chronic traumatic encephalopathy

B



C

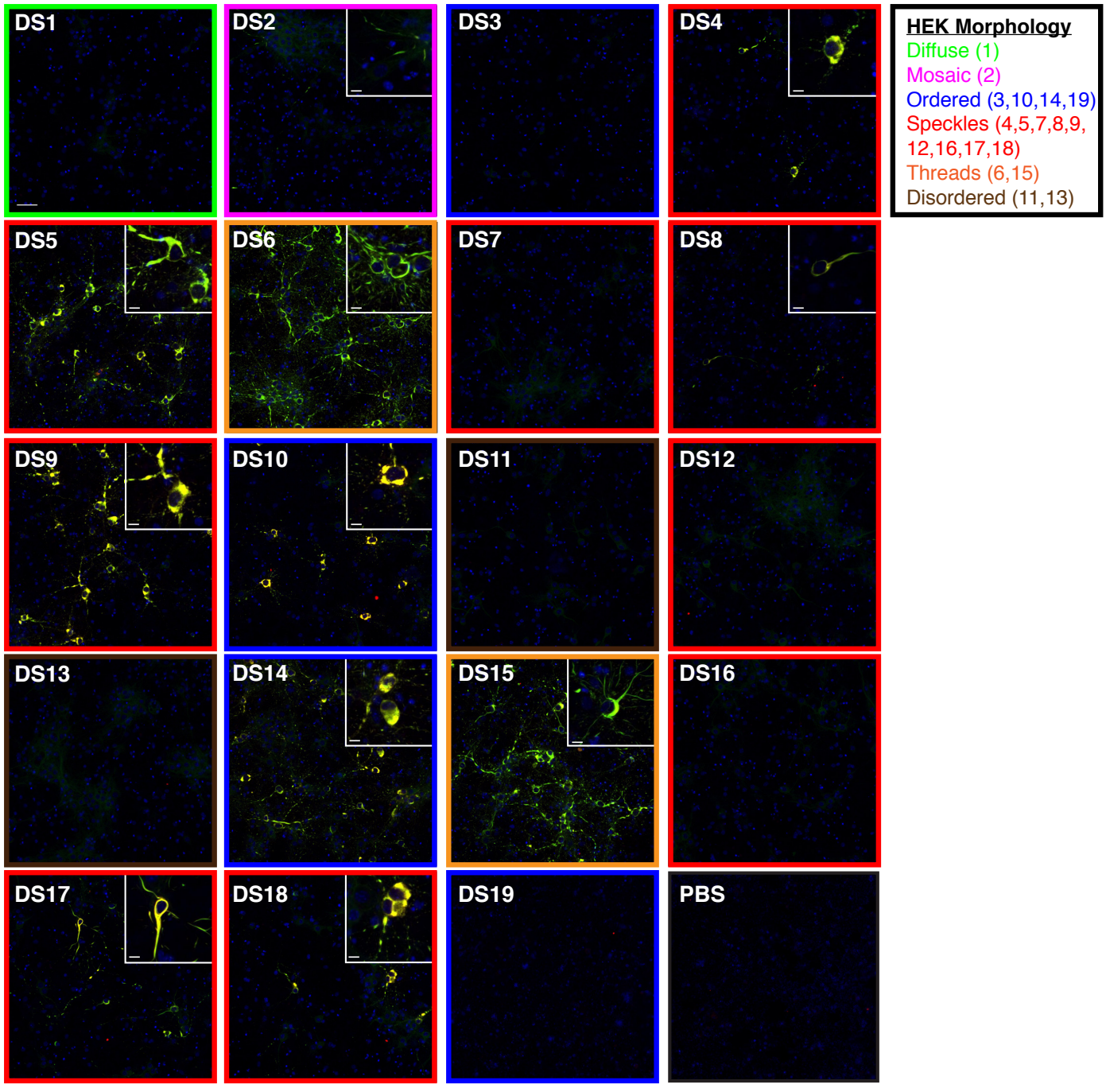


Figure S2, related to Figure 2. Seeding activity but not total tau correlates with toxicity *in vitro*

(A) Strain seeding activity correlates with toxicity. The number of aggregate-positive (FRET+) cells for a strain was plotted against its associated EC_{50} in the tau split-luciferase complementation assay. Strains that reached their half-maximal seeding at lower protein concentrations were associated with decreased cell growth.

(B) A significant correlation exists between cell growth (FRET+ cells) and seeding inflection point (the amount of lysate required for a strain to show significant seeding in the tau split-luciferase complementation assay).

(C) Densitometric analysis of tau in the total fraction suggests variability in the extent of total tau in the various strains. Error bars represent S.E.M. of biological quadruplicates.

(D) Total tau does not correlate with a strain's peak seeding ratio.

(E) No correlation was observed between a strain's total tau and its toxicity as assessed by relative growth of aggregate-positive (FRET+) cells.

(F) Normalized, blinded counts of strain morphology for DS1, 4, 6, 7, 9, 10, 11, and these strains 5 days and 8 days after transduction into the naïve DS1 cell line. Strains were classified as threads, ordered, disordered, or speckled as described in the methods.

(G) Representative images used for quantitation of the original strains and secondary polyclonal strains at 5 and 8 days post-transduction.

(H) Enlarged images of the original and transduced strains.

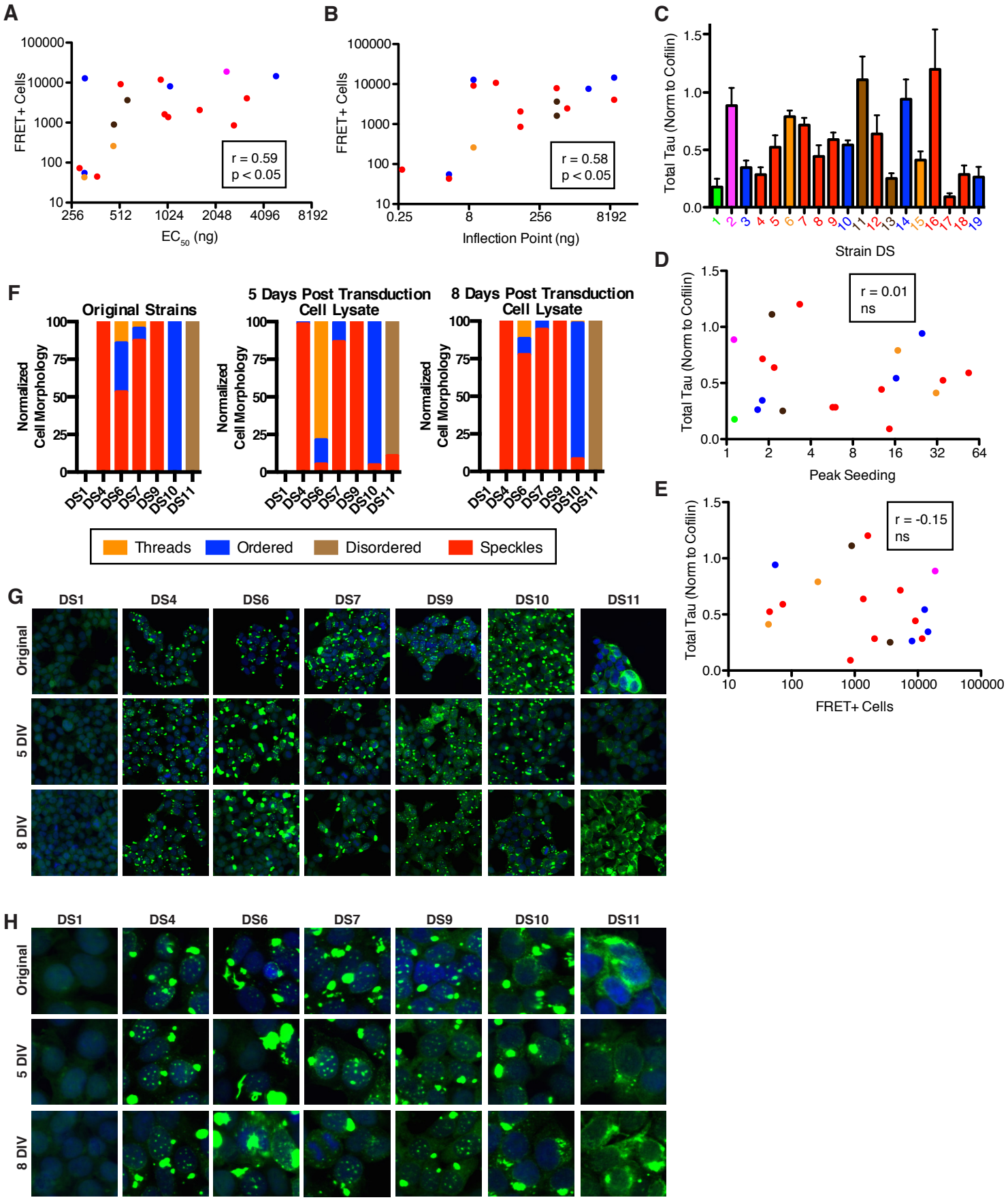

A
FRET+ Cells
100000
10000
1000
100
10
256
512
1024
2048
4096
8192
EC50 (ng)
r = 0.59
p < 0.05
B
FRET+ Cells
100000
10000
1000
100
10
0.25
8
256
8192
Inflection Point (ng)
r = 0.58
p < 0.05
C
Total Tau (Norm to Cofilin)
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Strain DS
D
Total Tau (Norm to Cofilin)
1.5
1.0
0.5
0.0
1
2
4
8
16
32
64
Peak Seeding
r = 0.01
ns
E
Total Tau (Norm to Cofilin)
1.5
1.0
0.5
0.0
10
100
1000
10000
100000
FRET+ Cells
r = -0.15
ns
F
Original Strains
5 Days Post Transduction Cell Lysate
8 Days Post Transduction Cell Lysate
Normalized Cell Morphology
100
75
50
25
0
DS1 DS4 DS6 DS7 DS9 DS10 DS11
Threads
Ordered
Disordered
Speckles
G
DS1 DS4 DS6 DS7 DS9 DS10 DS11
Original
5 DIV
8 DIV
H
DS1 DS4 DS6 DS7 DS9 DS10 DS11
Original
5 DIV
8 DIV

Figure S3, related to Figure 3. Strains induce unique tau pathology in various brain regions

(A) AT8 pathology present in the ipsilateral hippocampus of DS1-19. HEK Morphology refers to the inclusion appearance in HEK cells of strains inoculated into the hippocampus. Scale bars = 250 μm.

(B) Spread of AT8 tau pathology to the contralateral hippocampus and ipsilateral entorhinal cortex in DS5 and 9 (medium tangles), and DS6 and 15 (high tangles). Low levels of DS1 baseline staining can be observed in the ipsilateral EC, and is at the level expected for animals at this age. Scale bars = 250 μm for the whole hippocampus, and 50 μm for EC.

(C) DS10 and 14 show mossy fiber dots in the contralateral hippocampus. DS14 CA1 AT8 tangle-like pathology is much stronger than DS10. Scale bars = 250 μm for the whole hippocampus, and 50 μm for CA1 and CA3.

(D) DS18 shows grains throughout CA1, and wisps in the ipsilateral EC, showing the unique features induced by this strain can spread to distant regions. Scale bars = 250 μm for the whole hippocampus, and 50 μm for CA1 and EC.

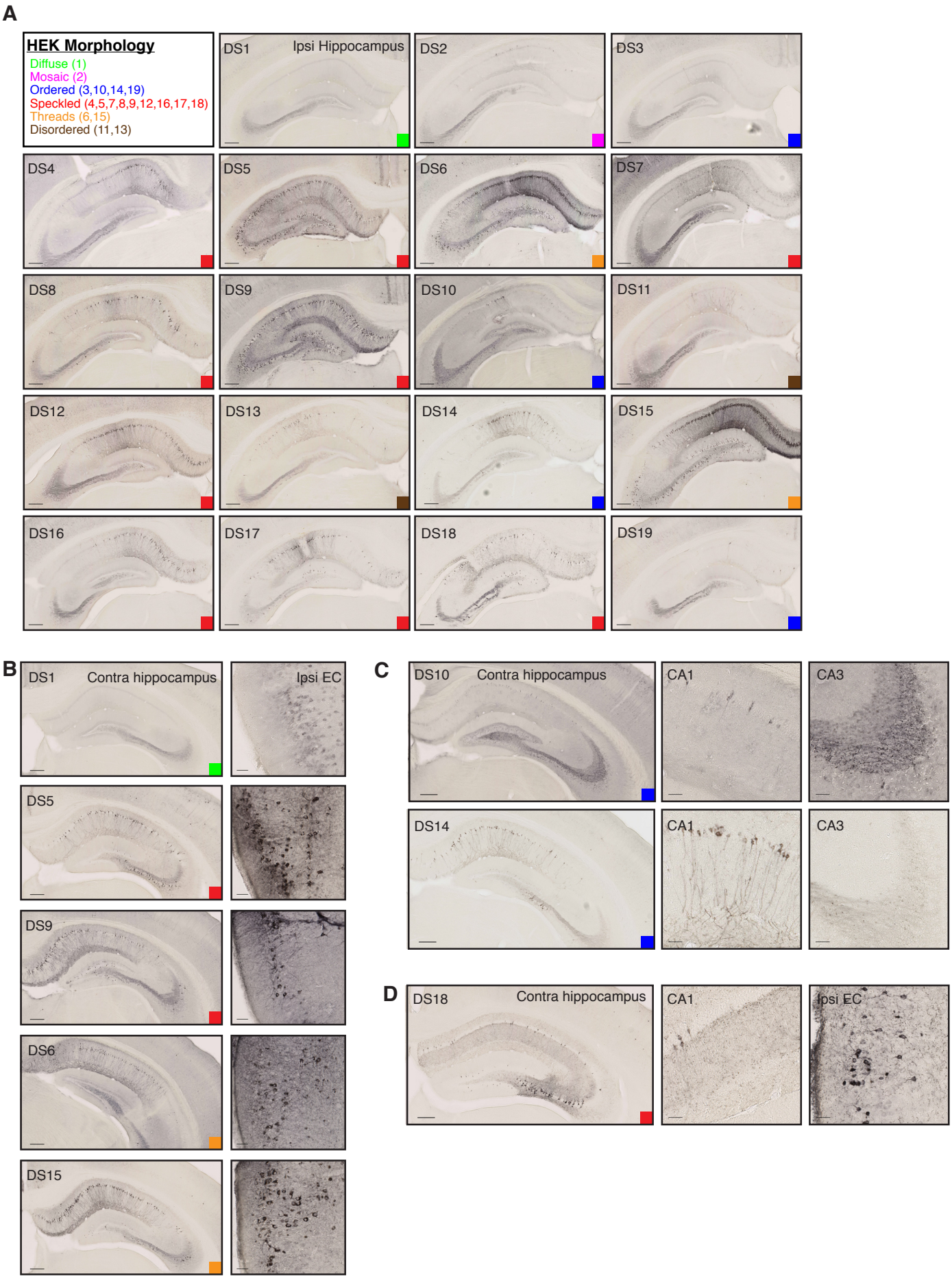

A
HEK Morphology
Diffuse (1)
Mosaic (2)
Ordered (3,10,14,19)
Speckled (4,5,7,8,9,12,16,17,18)
Threads (6,15)
Disordered (11,13)
DS1
Ipsi Hippocampus
DS2
DS3
DS4
DS5
DS6
DS7
DS8
DS9
DS10
DS11
DS12
DS13
DS14
DS15
DS16
DS17
DS18
DS19
B
DS1
Contra hippocampus
Ipsi EC
DS5
DS9
DS6
DS15
C
DS10
Contra hippocampus
CA1
CA3
DS14
CA1
CA3
D
DS18
Contra hippocampus
CA1
Ipsi EC

Figure S4, related to Figure 4. Certain strains reliably produce robust astrocytic pathology that increases over time.
(A) Quantification of the number of mice that displayed astrocytic tau pathology at 4, 8, or 12 weeks post-inoculation with different strains. Two experimental inoculations were assessed for astrocytic pathology (for strain panel and time course experiments, see **Supplemental Table 1 and 2**). Greater than 85% of animals inoculated with DS7 or 9 display astrocytic plaque-like pathology by 8-weeks. Other strains do not show any consistent plaque pathology until 12 weeks.

(B) Representative images of the astrocytic plaque-like pathology observed in 1/3 animals inoculated with DS12, 15, 16, and 18 at 8 weeks post-injection. Scale bars = 50 μm.

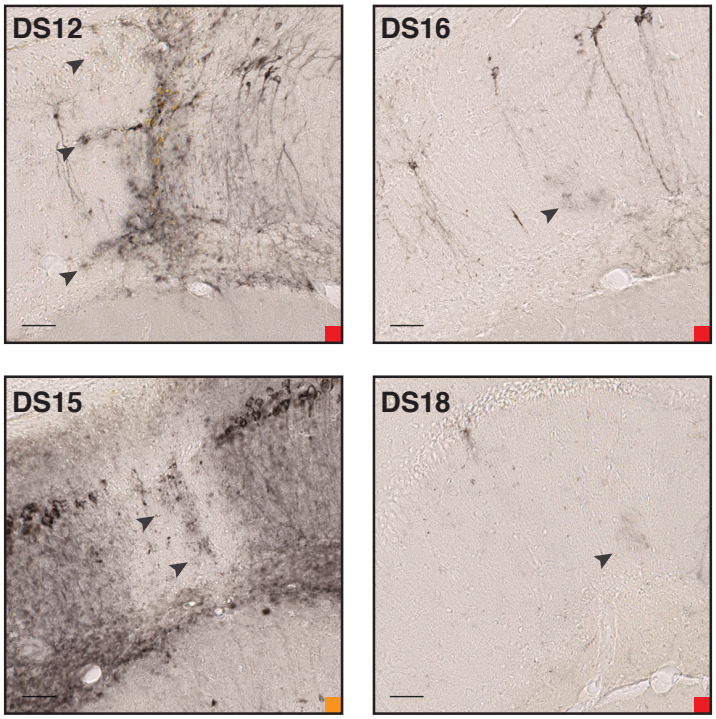
(C) Average number of plaques counted in ipsilateral hippocampus (also see Table S1). Several other strains begin to develop astrocytic pathology by 12 weeks, but the number of plaques is far lower than that observed in DS7 and 9.

(D) Images of age-matched PS19 mouse hippocampus and CA1 region, versus DS9, 12, and 16 inoculated hippocampi at 6 months post-inoculation. DS12 and 16 developed increased levels of astrocytic plaque pathology at this time point. Scale bars = 500um.

A

	Number of animals with astrocytic plaques		
	Weeks post inoculation		
Strain	4	8	12
DS1	0/4	0/10	0/5
DS2	-	0/3	-
DS3	-	0/3	-
DS4	0/4	0/4	4/5
DS6	0/5	0/6	4/6
DS6 1:10	0/5	1/4	3/4
DS7	0/5	7/8	6/6
DS9	2/5	6/7	6/6
DS9 1:10	3/4	4/4	4/4
DS10	0/5	0/5	0/6
DS11	-	0/3	-
DS12	-	1/3	-
DS13	-	0/3	-
DS14	-	0/3	-
DS15	-	2/3	-
DS16	-	1/3	-
DS17	-	0/3	-
DS18	-	1/3	-
DS19	-	0/3	-

B



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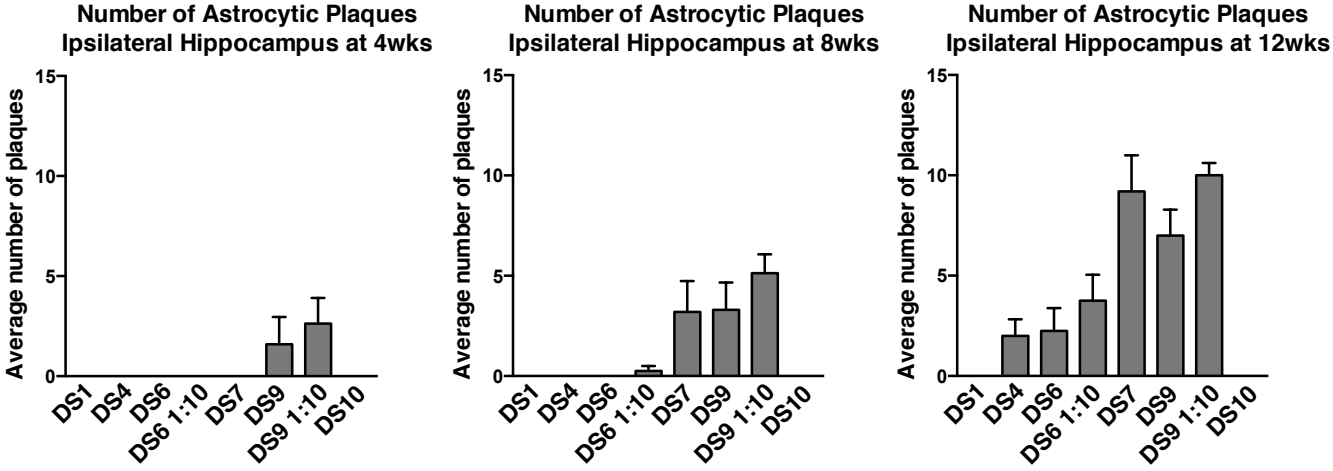


D

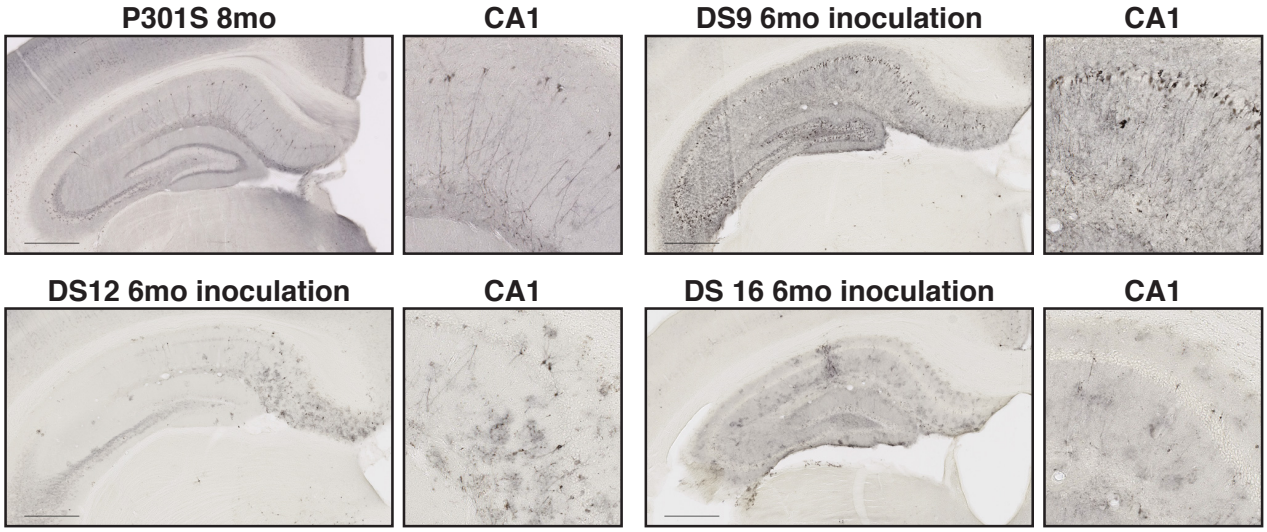


Figure S5, related to Figure 5. DS10 reliably produces mossy fiber dot pathology
(A) Mice inoculated with DS10 for the regional vulnerability experiments develop mossy fiber dots as expected in the ipsilateral and contralateral hippocampus. Scale bars = 250 μm for the whole hippocampus, and 50 μm for CA3.

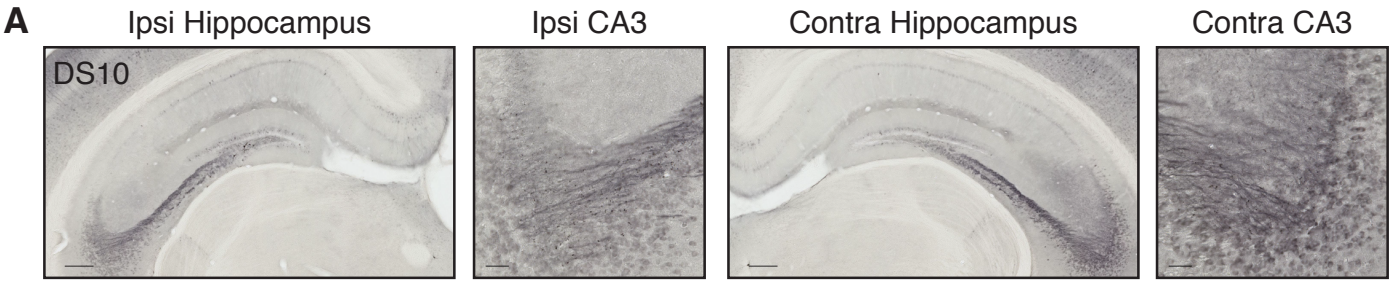

A
Ipsi Hippocampus
Ipsi CA3
Contra Hippocampus
Contra CA3
DS10

Figure S6, related to Figure 6. Strain Specific Microglial Phenotypes

(A) Ramified and rod microglia were quantified in the ipsilateral and contralateral hippocampus at 12 weeks post-inoculation.

(B) Rod microglia are significantly increased in all strains except DS7 in the ipsilateral hippocampus, but only in DS6 and 10 in the contralateral hippocampus. (* for $P \leq 0.05$; ** for $P \leq 0.01$; *** for $P \leq 0.001$; **** for $P \leq 0.0001$).

(C) Representative images of microglial pathology in mice 12 weeks post-inoculation. The rod microglial phenotype is apparent in several images (white arrowheads).

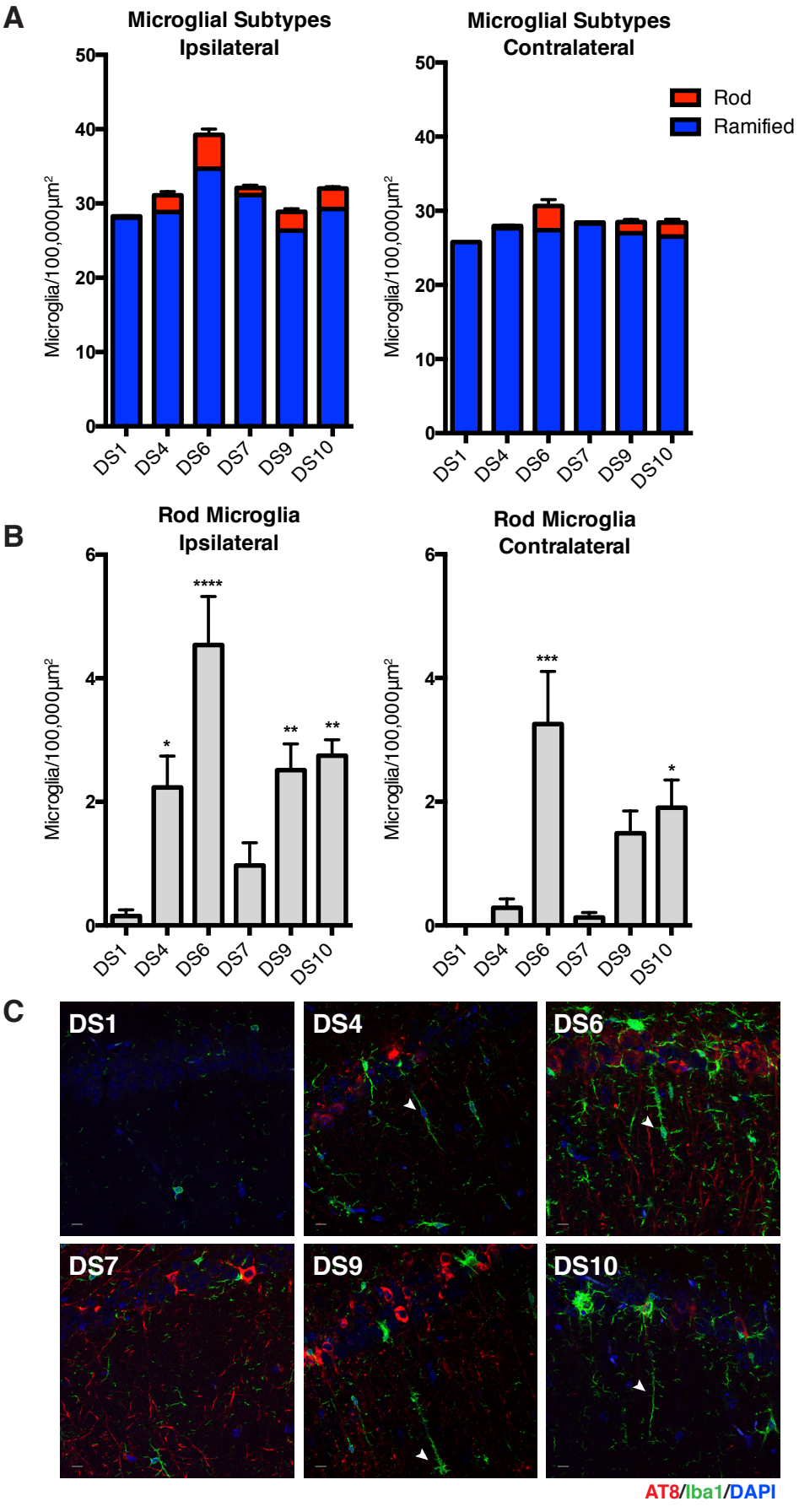

A
Microglial Subtypes
Ipsilateral
Microglial Subtypes
Contralateral
Rod
Ramified
Microglia/100,000µm²
50
40
30
20
10
0
DS1
DS4
DS6
DS7
DS9
DS10
B
Rod Microglia
Ipsilateral
Rod Microglia
Contralateral
6
4
2
0
*
**
**
****
***
*
C
DS1
DS4
DS6
DS7
DS9
DS10
AT8/Iba1/DAPI

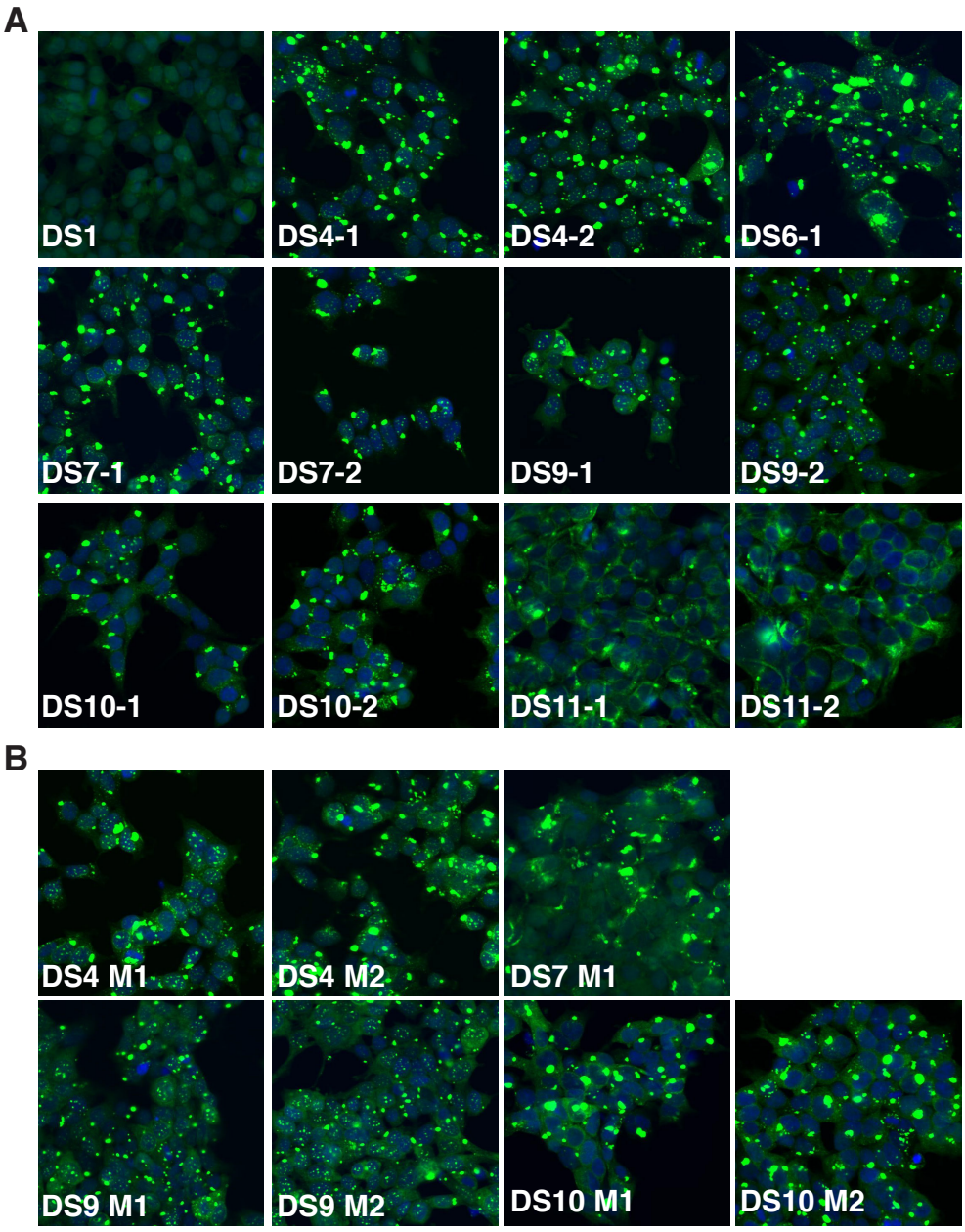
Figure S7, related to Figure 7. Monoclonal Cell Lines Maintain Original Strain Properties

(A) Representative images of monoclonal cell lines derived after single cell sorting of DS1 cells transduced with DS4, 6, 7, 9, 10, or 11 cell lysate.

(B) Representative images of monoclonal cell lines derived from mouse brain homogenate at 8 weeks post-inoculation of DS4, 7, 9, or 10.

(C) FRET-based seeding of original or secondary cell lines. Student t-tests were used to assess differences in seeding activity between the original and secondary cell lines (two-tailed, ns for $P > 0.05$; N/A if only one secondary cell line was isolated).

(D) Normalized blinded scoring of DS1 cells at 5 days post-transduction of mouse hippocampal homogenate. Transduced samples were from mice at 8 weeks post-inoculation with DS4, 6, 7, 9, or 10.


A
DS1
DS4-1
DS4-2
DS6-1
DS7-1
DS7-2
DS9-1
DS9-2
DS10-1
DS10-2
DS11-1
DS11-2
B
DS4 M1
DS4 M2
DS7 M1
DS9 M1
DS9 M2
DS10 M1
DS10 M2

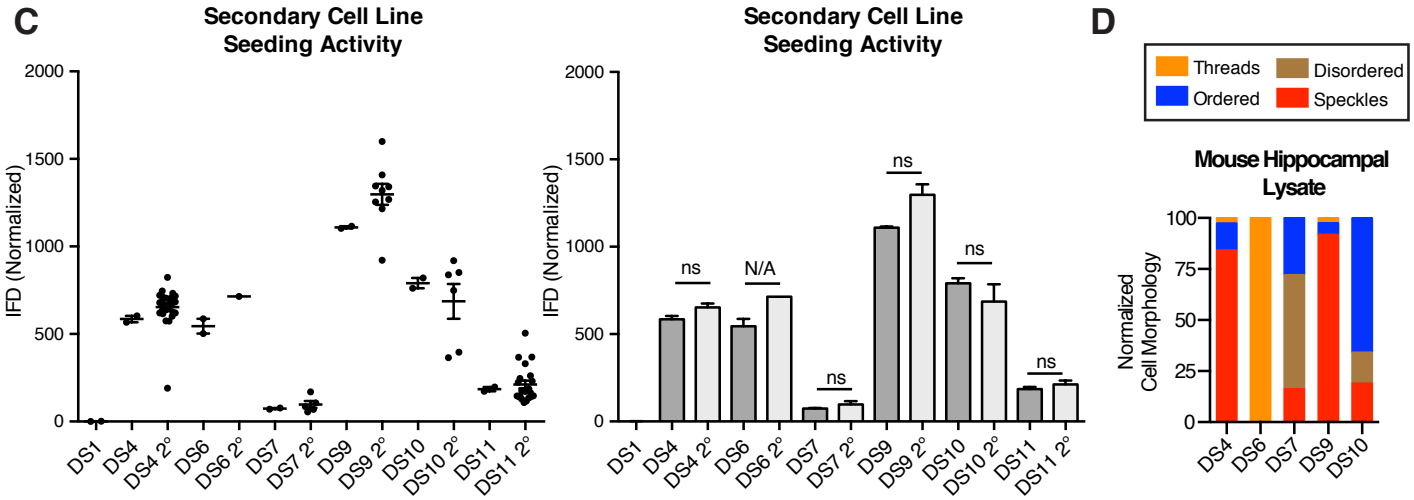

C
Secondary Cell Line Seeding Activity
IFD (Normalized)
2000
1500
1000
500
0
DS1
DS4
DS4 2°
DS6
DS6 2°
DS7
DS7 2°
DS9
DS9 2°
DS10
DS10 2°
DS11
DS11 2°
Secondary Cell Line Seeding Activity
IFD (Normalized)
ns
N/A
ns
ns
ns
ns
D
Threads
Disordered
Ordered
Speckles
Mouse Hippocampal Lysate
Normalized Cell Morphology
100
75
50
25
0
DS4
DS6
DS7
DS9
DS10

Table S1, related to Figure 6. Summary of mice used in time course inoculation experiment
List of experimental mice discussed in this study, including sex, age at time of surgery, and age at time of tissue collection.

Table S2, related to Figure 3. Summary of mice used in strain panel inoculation experiment
List of experimental mice discussed in this study, including sex, age at time of surgery, and age at time of tissue collection.

Table S3, related to Figure 5. Summary of mice used in regional vulnerability inoculation experiment
List of experimental mice discussed in this study, including sex, age at time of surgery, and age at time of tissue collection.

Table S1. Summary of Mice Used in Time Course Inoculation Experiment

Time Course	N (Total)	Male	Female	Age at time of surgery (months)	Age at time of sacrifice (months)
4 weeks					
DS1	4	2	3	2-3	3-4
DS4	5	2	3	2-3	3-4
DS6	5	2	3	2-3	3-4
DS7	5	2	3	2-3	3-4
DS9	5	2	3	2-3	3-4
DS10	5	2	3	2-3	3-4
DS6 1:10	5	2	3	2-3	3-4
DS9 1:10	4	2	3	2-3	3-4
8 weeks					
DS1	4	2	2	2-3	3-4
DS4	5	3	2	2-3	3-4
DS6	5	3	2	2-3	3-4
DS7	5	3	2	2-3	3-4
DS9	5	3	2	2-3	3-4
DS10	5	3	2	2-3	3-4
DS6 1:10	4	2	2	2-3	3-4
DS9 1:10	4	2	2	2-3	3-4
12 weeks					
DS1	5	2	3	2-3	4-5
DS9	6	2	4	2-3	4-5
DS10	6	3	3	2-3	4-5
DS4	5	3	2	2-3	4-5
DS6	6	3	3	2-3	4-5
DS7	6	3	3	2-3	4-5
DS6 1:10	4	2	2	2-3	4-5
DS9 1:10	4	3	1	2-3	4-5

Table S2. Summary of Mice Used in Strain Panel Inoculation Experiment

Strain panel	N (Total)	Male	Female	Age at time of surgery (months)	Age at time of sacrifice (months)
DS1	6	2	4	2-3	4-5
DS2	3	1	2	2-3	4-5
DS3	3	1	2	2-3	4-5
DS4	3	1	2	2-3	4-5
DS5	3	1	2	2-3	4-5
DS6	3	2	1	2-3	4-5
DS7	3	1	2	2-3	4-5
DS8	3	1	2	2-3	4-5
DS9	3	2	1	2-3	4-5
DS10	3	2	1	2-3	4-5
DS11	3	1	2	2-3	4-5
DS12	3	1	2	2-3	4-5
DS13	3	1	2	2-3	4-5
DS14	3	1	2	2-3	4-5
DS15	3	1	2	2-3	4-5
DS16	3	1	2	2-3	4-5
DS17	3	1	2	2-3	4-5
DS18	3	1	2	2-3	4-5
DS19	3	1	2	2-3	4-5

Table S3. Summary of Mice Used in Regional Vulnerability Inoculation Experiment

Regional Vulnerability	N (Total)	Male	Female	Age at time of surgery (months)	Age at time of sacrifice (months)
DS1	3	2	1	3-3.5	4-5
DS4	3	1	2	3-3.5	4-5
DS6	3	1	2	3-3.5	4-5
DS7	3	1	2	3-3.5	4-5
DS9	3	1	2	3-3.5	4-5
DS10	3	1	2	3-3.5	4-5
DS11	3	1	2	3-3.5	4-5

Supplemental Experimental Methods

Tau purification and fibrillization

Recombinant preparations of full-length (FL) 2N4R tau protein were prepared as previously described (Goedert and Jakes, 1990). pRK172 FL 2N4R tau was expressed in Rosetta (DE3)pLACI competent cells (Novagen) and tau was purified according to established protocols. After purification, tau was lyophilized using a FreeZone Plus Freeze Dry System (Labconco) and stored at -80°C as single-use aliquots. Prior to fibrillization, monomeric tau was re-suspended in 25 mM DTT for 45 minutes. The protein was then fibrillized at a final concentration of 8 μM in tau buffer (2.5 mM DTT/10 mM HEPES pH=7.4/100 mM NaCl/8 μM heparin, final volume of 200μL) at 37°C for 24 hours prior to addition to cells.

Cell culture

All HEK293T and HEK293 cell lines were grown in complete media: Dulbecco's Modified Eagle's Medium (DMEM) (Gibco) with 10% fetal bovine serum (HyClone) and 1% penicillin/streptomycin (Gibco). Cells were cultured and passaged at 37°C, 5% CO_2, in a humidified incubator. Dulbecco's phosphate buffered saline (Life Technologies) was used for washing the cells prior to trypsinization with 0.05% Trypsin-EDTA (Life Technologies).

Lentivirus production

Lentivirus was prepared as previously described (Araki et al., 2004; Sanders et al., 2014). HEK293T cells were plated at $1x10^6$ cells/well in 6-well plates. After 24 hours, cells were transfected with a three-component plasmid system for virus production: VSV-G (400 ng), PSP (1200 ng), FM5 (400 ng). DNA was mixed with 7.5μL TransIT-293 (Mirus) and 250μL OptiMEM for 15 minutes prior to addition to cell media. After 48 hours, media was harvested and spun at 500 x g for 5 minutes to remove debris. For generation of HEK293 cell lines, lentivirus-containing supernatant was stored at -80°C prior to addition to cells. For primary neurons, lentiviral supernatant was concentrated 50x using lenti-X concentrator (Clontech) according to manufacturer's protocol. The final concentrated virus was re-suspended in PBS with 25 mM HEPES (pH = 7.4).

Stable HEK293 cell line generation

Monoclonal HEK293 cell lines were generated that stably overexpress fusion proteins containing the tau repeat domain (amino acids 244-372 of the 2N4R isoform of tau) with various mutations (P301L, P301S, V337M) previously shown to cause familial tauopathy (FTDP-17) (Hutton et al., 1998). Two monoclonal HEK293 cell lines were previously described: Clone 1/DS1, which expresses tau RD(P301L/V337M)-YFP (Sanders et al., 2014) and the tau RD(P301S) FRET biosensor (ATCC CRL-3275), which expresses tau RD(P301S)-CFP and tau RD(P301S)-YFP (Holmes et al., 2014). To generate additional polyclonal lines, HEK293 cells were plated at 50,000 cells/well in a 12-well plate. After 18 hours, 300μL conditioned media containing lentivirus was added to the wells (RD LM FRET polyclonal: 150 μL tau RD(P301L/V337M)-CFP and 150 μL tau RD(P301L/V337M)-YFP; RD P301S split-luciferase polyclonal: 150μL tau RD(P301S)-Cluc and 150μL tau RD(P301S)-Nluc). For both polyclonal lines, cells were given 3 days to grow in the presence of virus. Cells were then re-plated in a 10 cm dish, grown to confluency, and stored in liquid nitrogen until use.

To generate monoclonal lines, the two polyclonal populations (RD LM FRET and RD P301S split-luciferase) were diluted sparsely in 10 cm dishes, so that there were fewer than 30 cells per dish. Cells were given seven days to grow into visible colonies. At this point, cloning cylinders (Bel-Art Products) were used to isolate single colonies, which were passaged to new wells. Monoclonal lines were serially amplified to confluency using 24-well then 6-well then 10 cm dishes. Upon reaching confluency in 10 cm dishes, monoclonal lines were frozen in liquid nitrogen until use. LM10 was the tenth monoclonal line selected from the RD LM FRET polyclonal, and was chosen due to its ability to differentiate toxic and non-toxic strains (described below). The RD P301S-split luciferase biosensor was selected due to its high signal to noise ratio in differentiating lysates with and without aggregates.

Lysate production for generation of strain library

Confluent cells from 6-well plates were harvested, pelleted, and stored at -80°C for the following cell lines (Sanders et al., 2014): Clone 1-derived (Clone 1, Clone 9, Clone 10), OFF1-derived (AGD2/mosaic, CBD3/disordered, CBD5/disordered, CBD3/speckles, AD1/speckles, PiD2/ordered). Cell pellets were thawed on ice and subsequently lysed in PBS with 0.05% Triton X-100 and a cOmplete mini protease inhibitor tablet (Roche) by triturating 10x and incubating at 4°C for 10 minutes. Sequential 5-minute centrifugations were then performed at 500 x g and 1000 x g to clarify the lysate. A Bradford assay (Bio-Rad) was performed on the supernatants, and protein concentrations were normalized to 5 µg/µL with addition of lysis buffer. Lysates were stored at -80°C prior to addition to cells.

To produce brain homogenates, the following samples were used: transgenic P301S mouse brain (age = 9 months), an Alzheimer's disease patient brain (patient AD1) (Sanders et al., 2014), and a late-stage chronic traumatic encephalopathy patient brain that was a generous gift from Dr. Ann McKee. For each, 0.4 gram sections were sonicated in 5 mL TBS with cOmplete protease inhibitors (Roche) using an Omni-Ruptor 250 probe sonicator at 30% power for 3-second pulses x 30 cycles. Crude homogenates were then clarified by centrifugation at 15,000g for 15 minutes. The supernatants were set aside, a Bradford was performed, and protein concentrations were normalized to 4 µg/µL with addition of TBS buffer. Homogenates were stored at -80°C prior to addition to cells.

Generation of a library of monoclonal strains

To generate a library of isogenic tau strains, several monoclonal tau RD(P301L/V337M)-YFP cell lines were examined for their ability to stably propagate a diversity of strains. Clone 1/DS1 (Sanders et al., 2014) was selected for its capacity to amplify both highly toxic strains and strains prone to sectoring, which can be attributed to its intermediate level of tau RD expression. Clone 1 cells were plated at 240,000 cells/well in 12-well plates. After 24 hours, cells were treated with 400 nM tau fibrils or 20 µg lysate/homogenate (see lysate production for generation of strain library), and prepared as transduction complexes (4µL lipofectamine-2000 (Life Technologies), protein source, and OptiMEM to 200µL volume). After 18 hours, cells were washed, trypsinized, and re-plated in a 6-well plate. At confluency (day 3), cells were diluted sparsely in 3 x 10 cm dishes (per condition) so that there were less than 30 cells per dish. Cells were given 9 days to amplify into visible colonies. At day 12, epifluorescence microscopy was used to find colonies featuring cells with inclusions and these were marked. Cloning cylinders (Bel-Art Products) were used to isolate up to six colonies per condition, and each individual colony was separately amplified to confluency in 12-well, then 6-well, and 10 cm dishes. At approximately day 30, cells were either plated on coverslips for fixation and confocal microscopy or were frozen down in single-use pellets at -80°C for analysis by seeding and protease digestion. A total of 90 monoclonal lines were examined. Based on preliminary analysis by inclusion morphology, seeding, and protease digestion, 21 were selected for rigorous analysis by both the aforementioned assays as well as sedimentation analysis and toxicity assays. Finally, 18 lines were suspected to be distinct strains and were designated DS2 to DS19 (DS = David/Sarah).

Fixation of cells for confocal microscopy

Cells were grown for 48 hours on coverslips in 24-well plates. Media was removed and replaced with 4% PFA for 15 minutes. PFA was removed and replaced with PBS. Cells were washed once with additional PBS and DAPI-stained for 10 minutes in 0.1% Triton-X. Stain was removed and replaced with PBS. Coverslips were mounted using Prolong Gold Antifade reagent (Life Technologies), sealed with nail polish, and placed at 4°C prior to confocal analysis.

Confocal analysis of strains based on inclusion morphology

For confocal microscopy, a Zeiss Axiovert 200M microscope was coupled to a Zeiss LSM 5 PASCAL system. For the collection of all images, a pinhole size of 0.8 µm was used. Representative images of each monoclonal cell line were taken and were scored based on inclusion morphology: diffuse (no aggregates), mosaic (loss of aggregated state with division), ordered (large, dense juxtanuclear inclusion with no nuclear aggregates), speckles (small juxtanuclear inclusion with numerous nuclear speckles), threads (long, fibril-like inclusions in

cytoplasm with rare nuclear speckles), disordered (diffuse aggregates that wrapped around nucleus with no nuclear speckles).

For blinded analysis of strain phenotype, images were collected with the In Cell Analyzer 6000 at 40x resolution with the assistance of the UTSW HTS Core facility. Images were coded and a blinded counter scored aggregate morphology according to the previously established guidelines discussed above (Sanders et al., 2014). Decoding was performed after completion of blinded counting, and morphology counts were normalized to total number of aggregate-positive cells.

Limited proteolysis

Lyophilized pronase (Roche) was re-suspended in PBS to a final concentration of 1 mg/mL and aliquots were snap-frozen and placed at -80°C. Cell pellets were thawed on ice and lysed in PBS with 0.05% Triton X-100 and a cOmplete mini protease inhibitor tablet (Roche) by triturating 10x and incubating at 4°C for 10 minutes. Sequential 5 minute centrifugations were then performed at 500 x g and 1000 x g to clarify the lysate. A Bradford assay (Bio-Rad) with BSA standard curve was performed and protein concentrations were normalized to 4 µg/µL with addition of lysis buffer. 60 µg (15 µL) of cell lysate was added to 15 µL of pronase (diluted in PBS) at a concentration of 60 µg/mL for a final volume of 30 µL and a final pronase concentration of 30 µg/mL. Cell lysates were digested at 37°C for one hour. Reactions were quenched by addition of 30 µL 2x sample buffer (4 µL BME, 11 µL 10% SDS, 15 µL 4x Laemmli buffer) followed by five minutes of boiling. 13 µL of each sample was loaded onto a NuPAGE 10% Bis-Tris gel (Life Technologies). Gels were run at 150 V for 70 minutes. Protein was transferred to Immobilon P (Millipore) using a semi-dry transfer apparatus (Bio-Rad). Membranes were blocked for 1 hour in 4% milk. Membranes were probed for tau using mouse monoclonal anti-tau 2B11 (0.7 µg/µL, Clontech) for 18 hours, washed four times with TBS-T, counter-probed with goat anti-mouse HRP (1:4000, GE Healthcare) for 1.5 hours, and were washed four additional times with TBS-T. Finally, membranes were exposed to ECL Prime Western Blotting Detection System (Fisher Scientific) for 2 minutes and were developed using a digital Syngene imager. For diluted strain limited proteolysis, 40 µg of strain cell lysate, or 20 µg of strain lysate mixed with 20 µg of HEK lysate were digested at 37°C for 90 minutes. In place of the relatively expensive 2B11 antibody, blots were developed using an antibody produced in bulk against amino acids 244-266 of wild type human 4R tau protein. This rabbit antibody was used at a working concentration of 1.35 µg/mL, and blots were otherwise developed as above.

Sedimentation analysis and densitometry of strain library

Cell lysates were prepared and clarified as described above (see Limited proteolysis) in biological quadruplicates for each strain. A Bradford assay (Bio-Rad) with BSA standard curve was performed to determine the protein concentrations for the 76 samples, normalizing each to 1.6 µg/µL. 1 mL of each was centrifuged at 186,000 x g for 60 minutes, with the remainder being set aside as the total fraction. After centrifugation, the supernatant fraction was placed aside and the pellet was washed with 1 mL lysis buffer. The ultracentrifugation step was repeated for 30 minutes, and the wash fraction was aspirated. Final pellets were re-suspended in 1 mL of 4% SDS/1% BME (in PBS) with the aid of five minutes of boiling. For each sample, 1 µg total (or equivalent volume of supernatant or pellet) was run on a NuPAGE 10% Bis-Tris gel (Life Technologies) at 150 V for 35 minutes. Protein was transferred to Immobilon P (Millipore) using a semi-dry transfer apparatus (Bio-Rad). Membranes were blocked for 1 hour in 4% milk and then probed overnight with rabbit polyclonal anti-tau ab64193 (1:5000, AbCam) and rabbit polyclonal anti-cofilin (1:28,000, Sigma). Following four washes with TBS-T, blots were counter-probed with goat anti-rabbit HRP (1:4000, Jackson Immunotherapy) for 90 minutes at room temperature. Finally, blots were washed an additional four times and membranes were imaged with a digital Syngene imager following exposure to ECL Prime Western Blotting Detection System (Fisher Scientific) solution for two minutes. Biological replicates were imaged separately, with four blots being developed at a time. Densitometric units were calculated using Syngene GeneTools software with manual band quantification. The brightness of each tau band (total, supernatant, pellet) was normalized relative to the signal calculated for the condition's associated total cofilin. Ratios were then averaged across biological quadruplicates.

Split-luciferase complementation assay

A monoclonal HEK293 cell line expressing tau RD (P301S)-Cluc and tau RD (P301S)-Nluc was generated (see Stable HEK293 cell line generation) and plated at 24,000 cells/well in 96-well plates. After 18 hours, when the cells were at 50% confluency, cells were transduced with cell lysate: indicated amounts of clarified cell lysate (see Limited Proteolysis) were added in transfection complexes with 0.4 µL lipofectamine-2000 (Life Technologies) to each well. 60 hours after addition of lysate, media was aspirated and replaced with luciferin solution (150 µg/mL D-luciferin potassium salt, Gold Biosciences, in Dulbecco's phosphate-buffer saline, Gibco). Cells were incubated in luciferin solution for two minutes at 37°C prior to reading luminescence with a Tecan M1000 fluorescence plate reader. Each condition was performed in biological quadruplicate with each replicate being performed on a separate plate to control for differences between plates. Seeding ratio was calculated relative to sham control for an individual plate. Seeding ratios were then averaged across quadruplicates and standard errors of the mean were calculated and plotted. Inflection point was defined as the amount of lysate required to achieve a 50% increase in luminescence relative to sham treatment. EC_{50} and peak seeding were calculated using a non-linear regression with one-phase decay fit.

Toxicity assay

A monoclonal cell line expressing tau RD (P301L/V337M)-CFP and tau RD (P301L/V337M)-YFP was generated and given the name LM10 (see Stable HEK293 cell line generation). LM10 cells were plated at 240,000 cells/well of 12-well plates. After 18 hours, wells were transduced with 20 µg clarified lysate (see Limited Proteolysis) in transfection complexes with 4 µL lipofectamine-2000. Each condition was performed in biological triplicate. After 24 hours, media was aspirated and cells were trypsinized and re-plated into 6-well plates. After 48 hours (i.e. 72 hours post-treatment), media was removed, cells were trypsinized, and pelleted, followed by resuspension in flow cytometry buffer (HBSS with 1% FBS and 1 mM EDTA). FRET-positive (aggregate-containing) and FRET-negative (aggregate-lacking) cells were gated according to published protocols (Furman et al., 2015; Holmes et al., 2014). Using a FACS Aria II SORP cell sorter (BD Biosciences), 7000 FRET-positive cells were collected in 1.4 mL media for each condition (DS2 to DS19). For the negative control (DS1), 7000 FRET-negative cells were sorted. 200 µL (i.e. 1000 cells) were plated in sextuplicate for each condition in 96-well plates. Cells were given 7 days to amplify. Cells were then harvested with 0.05% trypsin, pelleted, and fixed in 2% paraformaldehyde (Electron Microscopy Services) for 10 minutes. Cells were re-pelleted and resuspended in flow cytometry buffer. A MACSQuant VYB (Miltenyi) was used to perform FRET flow cytometry according to previously published protocols (Holmes et al., 2014), and total FRET-positive and FRET-negative cells were counted for each condition. Totals were averaged across technical sextuplicates and then averaged across biological triplicates. Error bars represent the standard error of the mean for biological triplicates.

Statistical analysis of *in vitro* correlations

For the correlation of toxicity, seeding, and sedimentation metrics, normality of the data was assessed using both D'Agostino/Pearson and Shapiro-Wilk tests. If both tests were passed, data sets were considered normal and a Pearson correlation was performed. Else, the data sets were not considered normal and a more conservative Spearman correlation was performed. Correlations were considered significant if p was less than or equal to 0.05.

Primary neuron culture and staining

Cortical and hippocampal neurons were isolated from E18.5 CD1 mice and cultured according to previous literature (Yano et al., 2014). Briefly, neurons were incubated with 0.5% trypsin in HBSS with glucose (1:6 dilution) for 20 minutes at 37°C. Subsequently, neurons were triturated and passaged through a 70 µm filter, followed by plating 100,000 cells per well on poly-D lysine- or polyethyleneimine (PEI)-coated plates for cortical or hippocampal cultures respectively. Neurons were treated at DIV3 with 2 µL of 50x-concentrated 1N4R tau(P301S)-YFP lentivirus (see Lentivirus production) per well of a 96-well plate. Neurons were subsequently treated at DIV6 with 10 µg of cell lysate or PBS. Neurons were examined for intracellular

aggregates each day with an epifluorescence microscope (Nikon Eclipse TI), and scored using a 0-5 scale (No seeding – Peak seeding). Neurons were fixed at 5, 8 or 11 days post lysate addition with a modified triton-X extraction to remove soluble tau protein (Volpicelli-Daley et al., 2011). Briefly, cells were incubated with 4% PFA/4% sucrose in PBS with 1% Triton-X 100 for 30 minutes. Cells were subsequently washed and stained with MC1 antibody (1:500; Peter Davies) in 10% NGS in PBS overnight at 4°C. Cells were washed and treated with anti-mouse IgG 546 (1:400, Life Technologies), and stained with DAPI. Cells were imaged with the In Cell Analyzer 6000 at 40x resolution with the assistance of the UTSW HTS Core facility.

Monoclonal Secondary Cell Line Isolation and Characterization

Cell lysate or mouse brain homogenate was transduced into the LM1 cell line. After four days in culture, single cells were sorted into 5 x 96 wells per condition. Cells were allowed to grow for 7-10 days prior to amplification into 24-well dishes, and subsequently expanded into larger dishes. Cell pellets were collected for seeding assay experiments. Monoclonal cell lines were plated on 96-well plates, fixed with 4% paraformaldehyde, and stained with DAPI. These lines were subsequently imaged with the In Cell Analyzer 6000 at 40x resolution with the assistance of the UTSW HTS Core facility.

Primary neuron seeding assay

Hippocampal neurons were plated at 75,000 cells per well on PEI-coated plates. At DIV3, cells were treated with 1µL of 50x concentrated tau RD(P301S)-CFP and 1µL of tau RD(P301S)-YFP lentivirus. AT DIV6, cells were treated with 10µg of cell lysate or an equivalent volume of PBS. Cells were subsequently incubated for four days. A MACSQuant VYB (Miltenyi) flow cytometer was used to assess FRET-positive neurons according to previously published literature (Furman et al., 2015; Holmes et al., 2014).

Cell lysate production for animal inoculation experiments

DS1-19 cell lines were grown in 3 x 10cm dishes until 90% confluency. Cells were trypsinized, re-suspended in media and centrifuged at 500 x g. Cell pellets were washed with 1x PBS and stored at -80°C until use. Pellets were thawed on ice and re-suspended in 1x PBS with cOmplete protease inhibitors (Roche) using an Omni-Ruptor 250 probe sonicator at 30% power for 30, 3 second cycles. The probe sonicator was washed with 100% ethanol and ddH_2O between cell lines. Strains were subsequently centrifuged at 1000 x g, normalized to 7µg/µl by Bradford assay (Bio-Rad) and stored as aliquots at -80°C until use.

Sedimentation analysis and densitometry of injected lysates

Sonicated and clarified cell lysate was thawed on ice, and diluted to 2 µg/µL in 1x PBS with cOmplete protease inhibitors (Roche). Samples were subdivided into a total protein and ultracentrifuge (UC) aliquots. The UC aliquots were centrifuged at 186,000 x g for 90 minutes. Supernatant was removed and stored with the total lysate aliquot at -80°C until use. Pellets were washed with 1x PBS and centrifuged at 186,000 x g for an additional 30 minutes. Pellets were re-suspended to their original volume. SDS-PAGE was performed on total, soluble or insoluble fractions of each cell line (1:2:1 ratio) using 5-20% gradient acrylamide gels. Gels were transferred as described above. Membranes were cut at the 20kDalton ladder mark and incubated at 4°C overnight with a rabbit polyclonal anti-tau antibody (1:4000, Abcam ab64193) or a rabbit polyclonal anti-cofilin antibody (1:4000; Sigma). Anti-rabbit ECL HRP conjugated secondary antibody (1:4000, GE Lifesciences) was added for one hour, and blots were developed using ECL Prime Western Blotting Detection System (Fisher Scientific). Washes were performed as described above. Densitometry was performed by measuring the mean grey value (mgv) of bands with ImageJ, and normalizing to cofilin mgv. Samples were averaged across biological triplicate. A one-way ANOVA with Bonferroni's multiple comparisons correction was performed by comparing DS1-1 to each sample. A one-way t-test was performed to directly compare DS10 and DS4.

Animal maintenance

We obtained transgenic mice that express 4R1N P301S human tau under the murine prion promoter {Yoshiyama:2007bf} from Jackson Laboratory, and maintained them on a B6C3 background. Transgenic mice and wild-type littermates were housed under a 12 hour light/dark cycle, and were provided food and water *ad libitum*. All experiments involving animals were approved by the University of Texas, Southwestern institutional animal care and use committee.

Inoculation experiments

P301S mice were anesthetized with isoflurane and kept at 37°C throughout the inoculation. Mice were injected with separate 10 µL gas-tight Hamilton syringes for each strain at a rate of 0.2 µL per minute. For strain panel and time course experiments (Figures 3.5, 3.11, 3.13), animals were inoculated with 10 µg (1.428 µL) of cell lysate in the left hippocampus (from bregma: -2.5 mm posterior, -2 mm lateral, -1.8 mm ventral). For the regional vulnerability experiments (Figure 3.9), animals were injected at six sites (x, y, z from bregma: sensory cortex: -2.75, -0.2, -0.5; caudate/putamen: -2.75, -0.2, -2.8; visual cortex: -1.9, -2.9, -0.45; hippocampus: -1.9, -2.9, -1.4; thalamus: -1.9, -2.9, -4; inferior colliculus: -1, -5.6, -1.5) with 5 µg of lysate per region.

Animal tissue collection

P301S or WT mice were anesthetized with isoflurane and perfused with chilled PBS + 0.03% heparin. Brains were post-fixed in 4% paraformaldehyde overnight at 4°C and then placed in 30% sucrose in PBS until further use.

Histology

Brains were sectioned at 50µm using a freezing microtome. Slices were first blocked for one hour with 5% milk in TBS with 0.25% Triton X-100 (blocking buffer). For DAB stains, brain slices were incubated with biotinylated AT8 antibody (1:500, Thermo Scientific) overnight in blocking buffer at 4°C. Slices were subsequently incubated with the VECTASTAIN Elite ABC Kit (Vector Labs) in TBS prepared according to the manufacturer's protocol for 30 minutes, followed by DAB development using the DAB Peroxidase Substrate Kit with the optional nickel addition (Vector Labs). Slices were imaged using the Olympus Nanozoomer 2.0-HT (Hamamatsu). For astrocyte staining, slices were permeablized in 0.25% Triton X-100 in TBS, followed by blocking with 3% milk and 10% normal goat serum (NGS) for 30 minutes, followed by incubation with AT8 (1:500, Thermo Scientific) and GFAP (1:500, Dako) overnight at 4°C. For microglial staining, slices were treated with AT8 as above, and Iba1 (1:500, Dako). Slices were incubated with 1:500 anti-mouse 488 and anti-rabbit 546 antibodies for astrocyte staining (Life Technologies). Slices were imaged followed by imaging using a Zeiss LSM780 inverted confocal microscope. For microglial staining, slices were incubated in 1:500 anti-mouse 546 and anti-rabbit 488. Slices were imaged using a Zeiss Axioscan.Z1 at 40x resolution, or with a Zeiss LSM780 inverted confocal microscope.

Quantification of Tau pathology

Images of AT8 DAB stained slices were collected as above. A blinded analysis of the level of tau pathology was performed using a semi-quantitative 0-3 scale (no pathology, mild, moderate, and severe, respectively) as previously reported (Lace et al., 2009). Briefly, images of each brain slice were randomized and blinded to the scorer. Individual brain regions in each slice were assessed for AT8 positive neuronal inclusions. Scores were recorded unless a slice was damaged such that the region of interest was not present in the slice. The level of pathology was averaged among biological replicates for each region within a condition. For the regional vulnerability experiments (Figure 5), pathology was normalized by subtracting the average DS1 pathology in a region, and heat maps were generated with discrete bins for different pathology levels as described within the figure. For the time course experiments (Figure 7), averages were plotted as a heat map with a gradient from 0 to 3 as reported in the figure. Heat maps were generated using MATLAB.

Quantification of Iba1 microglial pathology

Slices from mice 12 weeks after inoculation with DS1, 4, 6, 7, 9, or 10 were stained for Iba1 as above. Hippocampi at the level of the injection site were outlined as a region of interest, and microglia were quantified in a blinded fashion for cellular morphology within this region of interest. Microglia were scored as ramified, rod, or activated non-rod morphologies. Microglial counts were averaged within a condition, and normalized to total area assessed.

P301S FRET Flow Seeding assay

Bilateral hippocampus, sensory cortex, or thalamic 1mm punches were isolated from mice injected with DS1, 4, 6, 7, 9, 10 or diluted DS6 and DS9, 8-weeks post inoculation. Samples were placed into 1xTBS at a dilution of $1mm^3$/mL (v/v) and sonicated with a water bath sonicator (Qsonica Q700MPX with chiller and tubing set) at 4°C at 50% power for 30 minutes. Seeding assays were performed as previously described (Furman et al., 2015; Holmes et al., 2014). Briefly, 2.5 µL of each sample was added in triplicate to wells of a 96-well plate onto the tau RD(P301S) FRET biosensor cell line. Cells were incubated for 48 hours and then trypsinized, fixed with 2% PFA in 1x PBS, and incubated in flow cytometry buffer. A FACS LSRFortessa SORP was used to perform FRET flow cytometry as previously described (Furman et al., 2015; Holmes et al., 2014). Integrated FRET density (IFD = percent cells positive for FRET multiplied by the median fluorescent intensity of FRET-positive cells) was averaged for every condition and normalized to lipofectamine-2000 (sham) IFD. A one-way ANOVA with Bonferroni corrections was performed for each region by comparing DS1 ipsilateral IFD to all other conditions within that region. For seeding of secondary monoclonal lines, the original monoclonal cell lines and secondary cell lines were prepared in PBS with 0.05% Triton X-100 with cOmplete mini protease inhibitors as described above. FRET biosensor cells were incubated with 2 µg of lysate per well, and cells were harvested at 24 hours post-transduction. Two separate cell pellets were prepared to assess the seeding activity of the original strains. A two-tailed student t-test was performed to compare seeding activity between the original cell line samples and secondary cell lines (ns for $P \geq 0.05$).

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